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PHOTOSYNTHESIS

James A. Bassham and M. Calvin

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ABSTRACT

The overall process of photosynthesis involves a number of interconnected processes. These processes, which are cyclic with respect to both energy and material, are related at some points to well-known respiratory processes. The carbon-reduction cycle in photosynthesis is now known in detail. All enzymes involved in this cycle have been isolated and the sources of energy required for its operation have been identified in terms of reducing agents and "high-energy" phosphate. These sources of energy are derived ultimately from absorbed light energy which brings about the photolysis of water. Possible mechanisms for this photolysis and for the transfer of energy from the photolysis products to the carbon-reduction cycle are discussed here. Experimental data, in the form of quantum efficiency measurements, are presented and partially confirm the theories proposed for the mechanisms of energy transfer. A diagram of the complete process of photosynthesis containing the several cycles and their relations is presented.

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During the past decade, considerable advance in the understanding of the complex process of photosynthesis has been realized. This achievement has resulted both from the use of new methods of investigation and from the stimulation of interest, partly as a result of these new techniques, which has led to very widespread participation in the study of this problem throughout the scientific world. Fortunately, compilation and discussion of this immense amount of work[46, 47, 34, 65, 26, 27, 17] -- together with review articles of the most recent work[41, 36, 30] -- have generally kept pace with the work itself. In this paper, therefore, no responsibility for a complete inclusion of published work is assumed, but rather, an attempt is made to present some current opinions regarding selected aspects of photosynthesis, together with some speculations in areas that may be expected to prove fruitful in the near future.

From the discoveries of recent years, it has become increasingly apparent that photosynthesis includes a number of cyclic processes coupled to one another in such a way that there is a continuous flow of energy from one cycle to another, resulting in the conversion of light energy into the potential energy of new chemical bonds. These cyclic processes, while similar to better-known cyclic processes occurring during respiration in plants and animals, do not seem to be in any instance simple reversals of respiratory cycles, but do appear to have many points of contact with respiratory reactions, including common intermediates and enzymes. These points of contact may well lead to interaction between the two processes that could alter the course of each.[18] It appears that the organization of the green plant, both structural and enzymatic, may provide mechanisms for partially preventing such interactions where they would prove deleterious to the efficiency of the photosynthetic reaction, and possibly permitting or even inducing them where interaction is beneficial.

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1. Function of the Chloroplast

One such device for isolating photosynthesis from respiration is the chloroplast itself. It appears likely that the entire reaction of photosynthesis, from the absorption of light, carbon dioxide, and water to the formation of various end products, occurs in the chloroplasts. This probability was indicated long ago by the observed formation of starch granules in chloroplasts. (However, starch and other high-molecular-weight compounds are also formed outside the chloroplasts from simple sugars, amino acids, and other low-molecular-weight compounds which diffuse out of the chloroplast.) It has long been known that isolated chloroplasts can, under suitable conditions, retain the ability to evolve oxygen at rates comparable to those of photosynthesis, and at the same time, transfer reducing power to a suitable oxidizing agent. Efforts to demonstrate CO_2 reduction with isolated chloroplasts were generally unsuccessful in the past. However, Gerretsen[32] found a decrease in the oxidation reduction potential in illuminated chloroplast suspensions when they were supplied with carbon dioxide and concluded that there might be an uptake of carbon dioxide, though to only about 3% of the rate of photosynthesis. Boychenko and Baranov[7] have demonstrated the incorporation of carbon dioxide into reduced organic compounds by isolated chloroplasts under illumination. This incorporation was determined through use of carbon-14-labeled CO_2, providing a very sensitive method for detecting reduced carbon. This result recently has been confirmed by Arnon et al.[2] The rate of carbon reduction in isolated chloroplasts compared with that in a corresponding amount of photosynthesis in intact leaves was not more than 0.5%, and many of the intermediates of the carbon-reduction cycle have not yet been found. In any event, if it turns out that the rate is small compared with photosynthesis and that only some steps involved in the complete cyclic reduction during photosynthesis take place in the isolated chloroplasts, these facts may be taken merely as an indication that some of the factors involved in the rather complex reduction cycle have been lost from the chloroplasts during their isolation, or that only limited amounts of the necessary enzymes are carried down with them.

We could think of the chloroplast as a container of a complex arrangement of enzymes and cofactors, some of which are lost when the chloroplast is removed from the cytoplasmic environment of the cell. Possibly such loss could be prevented if the chloroplasts could be preserved throughout their isolation in a solution exactly duplicating that contained in the cell. Perhaps it would be worth while to prepare such a solution by disrupting cells and removing chloroplasts and cell-wall fragments by centrifugation. This solution could then be used during the preparation of chloroplasts from fresh cells.

The difference between the susceptibility to inactivation during chloroplast isolation displayed by the carbon-reducing apparatus and that found with the system for the photolysis of water can be considered as a difference in susceptibility to loss of factors or disruption of organization of various systems. It appears that the enzymatic apparatus for the absorption of light, the conversion of light energy to chemical energy, the decomposition of water by this energy, and the formation of oxygen and reducing power are all rather resistant to inactivation provided relatively simple buffer solutions

are employed in the isolation of the chloroplasts. This seems to indicate the presence of an organized enzymatic apparatus, perhaps with microscopically large and intricate structure and with all necessary cofactors and prosthetic groups firmly attached. Such a view is consistent with the apparent structure of the grana and lamellae as revealed by electron microscope studies.[40, 57]

The carbon-reduction cycle, however, must be easily inactivated or separated during preparation of isolated chloroplasts. This may indicate that the enzymes involved in this cycle are not part of an organized structure, except so far as the chloroplast structure tends to enclose these enzymes and retain within itself a space with high reducing potential.

It seems likely that this reducing power does not diffuse out of the chloroplast. If it did, one might expect greater inhibition of respiration in the light than is actually observed in cells that contain chloroplasts.[12, 31] It is significant that inhibition of respiration during photosynthesis is most pronounced in some organisms that do not contain organized chloroplasts,[11, 13] and this fact again indicates that one role of the chloroplast structure may be to retain reducing power at a high level for photosynthesis. Another bit of evidence relating to this possibility is the observation that the conversion of photosynthetic intermediates to respiratory intermediates is inhibited in the light.[18] This may be attributed to the reducing condition within the chloroplast. In this case, the inhibition is believed to be due to the reduction of a specific cofactor rather than to a general reduction of metabolic intermediates which may otherwise enter the oxidative pathway.

The mechanism by which the chloroplast may retain the reducing power is not entirely clear, since such carriers of reducing power as TPNH may be expected to diffuse out of the chloroplast rather freely. One possibility is that the primary carrier of the reducing power is itself a protein complex resistant to dialysis. Thus some type of metalloprotein may carry the reducing power. Another possibility is that reduced thioctic acid, a dithiol, may be a carrier of reducing power and may be attached by its carboxyl group to form a part of a lipid. It may be simply that the enzymes that reduce CO_2 in the chloroplast are so active that the reducing power is largely used before it can diffuse out of the chloroplast.

The other reagent requirement for the reduction of CO_2 at photosynthetic rates seems to be a high level of ATP, as is seen later in this discussion. Bradley(Dan F. Bradley, private communication) finds that the level of ATP is higher in the dark than in the light if the plant is in an atmosphere of 4% CO_2 in air, lower in the dark than in the light if the atmosphere is nitrogen. Strehler[54] finds that there is an increase in the level of ATP in the cell upon turning on the light after a period of darkness. This increase occurred during the induction period when the rate of carbon reduction had not yet reached its maximum value. These facts may be explained in the following way: In the dark with O_2, respiration and the production of ATP by some of the energy available from respiration proceed in both the chloroplast and the space outside. When the light is turned on the rate of production outside the chloroplast is not immediately affected, but inside the chloroplast there is a combination of several effects. Normal dark respiration ceases owing to the production of reducing power, and in

particular reduced thioctic acid,[18] while at the same time the production of ATP through energetic coupling of the recombination of some photochemically produced oxidizing and reducing agents begins.[4] The result of these various rate changes is an initial increase in the level of ATP. As the rate of CO_2 reduction increases, the demand for ATP increases. This results in a decrease in the level of ATP in the chloroplast, perhaps to a value much less than during the dark time, and ATP begins to flow into the chloroplast from the cellular space outside. In an atmosphere of N_2, on the other hand, respiration ceases in the dark, leading to a lower level of ATP in both chloroplast and cytoplasm, when the light is but turned on some ATP is formed in the chloroplast, owing to the oxidation (back reaction) of some of the photochemically generated reducing power by O_2 liberated from photosynthesis, or by an intermediate oxidant. The formation of ATP by the oxidation of an intermediate reductant (i.e. TPNH) is known from studies of oxidative phosphorylation. That some of the ATP was formed during the transfer of the electrons between intermediate reductant (TPNH) and intermediate oxidant (i.e. metalloflavin, and (or) cytochromes) is also an integral part of this process, although ultimately the electrons ($[H]$) are transferred to molecular O_2. The formation of ATP by the recombination of photoproduced intermediate reductants and oxidants before and without the possibility of the intervention of molecular oxygen has been experimentally observed by Frenkel,[28] using the plastids isolated from purple bacteria, which are incapable of making molecular O_2. A similar observation has been made by Arnon[2] and co-workers using specially prepared whole chloroplasts from spinach. In the latter case it was necessary to add other redox systems to replace molecular O_2, such as the naphthoquinone related to vitamin K, or ascorbic acid. In addition some participation of added FMN was demonstrated.

In regard to the location of the processes of photosynthesis, then, the chloroplast or its immediate environs seems to be the unique location of all important parts, and may provide a container or matrix for the complex system of enzymes and cofactors involved in the carbon-reduction cycle as well as a support for the pigment and enzyme structure which carries out energy conversion and decomposition of water.

2. The carbon-reducing enzymes

Two powerful tools have been brought to bear during the past decade on the problem of the path of carbon dioxide reduction during photosynthesis: tracer elements and paper chromatography. It is unlikely that our present degree of knowledge of carbon reduction would be anywhere near as far advanced had either of these tools been missing. In any event, it is now possible to trace the entire path of carbon reduction from the entry of carbon dioxide into the plant cell to the formation of sugars and other end products.[4] The essential steps in this process are

(a) the carboxylation of a sugar phosphate, ribulose diphosphate, to give two three-carbon molecules, both of which are probably phosphoglyceric acid (see discussion later in this section);

(b) the reduction (with the aid of ATP) of phosphoglyceric acid by reducing agents formed from water by the photochemical reactions; and

(c) the rearrangement of most of the molecules of the reduction product, phosphoglyceraldehyde, to give (with the aid of ATP) more ribulose diphosphate for continued carboxylation, with a smaller part of the sugar phosphates being drained off as end products.

The individual steps in this process are shown in Fig. 1, together with the enzymes believed to be involved. The carbon balance of this system is indicated in the following scheme.

$$3\ C_5 + 3\ CO_2 \longrightarrow 6\ PGA,$$

$$6\ PGA \xrightarrow[6\ ATP]{12[H]} 6\ C_3,$$

$$2\ C_3 \longrightarrow C_6,$$

$$C_6 + 2\ C_3 \longrightarrow C_5 + C_7,$$

$$C_7 + C_3 \longrightarrow 2\ C_5,$$

$$12[H] + 3\ CO_2 \xrightarrow{9\ ATP} C_3 + 3\ H_2O.$$

A slightly different version of the sugar rearrangement might be proposed in which different reactions replace those between fructose-6-phosphate and glyceraldehyde phosphate by transketolase to give ribulose-5-phosphate and a four-carbon aldose, which then combines with dihydroxyacetone phosphate (aldolase?) to give sedoheptulose-1, 7-diphosphate. The modified version would postulate that two molecules of fructose-6-phosphate are split and recombined by transketolase and transaldolase to give directly sedoheptulose-7-phosphate and ribulose-5-phosphate. Although it is not possible at present, to choose unequivocally between these two possibilities evidence obtained from degradation of the various radioactive sugar phosphates obtained from soybean leaves exposed to $C^{14}O_2$ for a very short time indicate that the original proposal (as shown in Fig. 1) is correct. These degradation results are shown in Table I, which is derived from Table I of Reference 4 by assuming the same C^{14} in carbons Nos. 1, 2, and 6 as that found in Carbon 7.

In either sugar rearrangement version carbons Nos. 4 and 5 of sedoheptulose are derived from Carbons 3 and 4 of fructose respectively. In the original version (Fig. 1), however, Carbon 3 of sedoheptulose is derived from Carbon 1 of glyceraldehyde phosphate directly, while in the modified version Carbon 3 of sedoheptulose is derived from Carbon 3 of fructose and therefore should have the same labeling at all times as Carbon 4 of the sedoheptulose. Since this is not the case, the original version of the rearrangement seems more likely, especially since at very short exposures of the plant to $C^{14}O_2$, Carbon 3 is much more highly labeled than Carbon 4, as would be expected if it were derived from Carbon 1 of the more recently formed dihydroxyacetone phosphate.

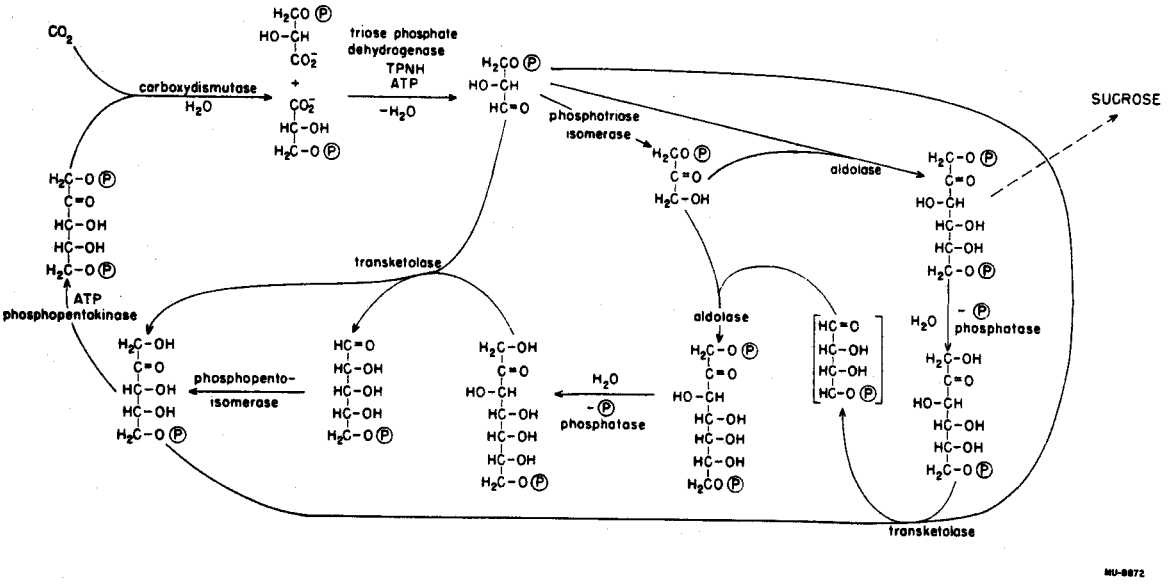


Fig. 1. The path of carbon in photosynthesis.

Table I

Distribution of C^{14} in Sedoheptulose

Isolated from Soybean Leaf

	Time of exposure to $C^{14}O_2$	
	0.4 sec	0.8 sec
H_2C-OH	0	2
C=O	0	2
HO-C-H	33	39
HC-OH	8	18
HC-OH	49	38
HC-OH	0	2
H_2C-OPO_3H^-	0	2

It is to be noted that the difference in labeling between Carbons 3 and 4 of fructose (hence 4 and 5 of sedoheptulose) was explained as arising from the formation of dihydroxyacetone phosphate (from which Carbon 3 of fructose is derived) subsequent to the formation of glyceraldehyde phosphate (the precursor of Carbon 4 of fructose).

Of all the enzymes indicated in Fig. 1 only two, the enzyme for the carboxylation of RuDP and the enzyme required for the phosphorylation of ribulose monophosphate, have not been found so far in tissues other than green plants. The preparation of the carboxylation enzyme in a cell-free extract has been reported by Quayle and co-workers,[45] while Weissbach et al. have isolated an enzyme preparation which causes the phosphorylation of RuMP to RuDP.[63] It appears that the phosphorylation and the carboxylation may be unique steps in the carbon-reduction cycle. If these steps are unique to photosynthesis, they may be so only because they either require enzymes not found outside green plants, or because the particular concentrations of metabolites within the chloroplast are required if these reactions are to proceed at a finite rate. Some evidence bearing on this point has been obtained in this laboratory. The purple photosynthetic bacterium, Rhodopseudomonas, not only is capable of photoreducing CO_2 with H_2, but it can also reduce CO_2 in the dark with H_2 or with organic substrates provided O_2 is present.[58, 53] This is usually spoken of as an energetic coupling of some of the energy released in the reaction

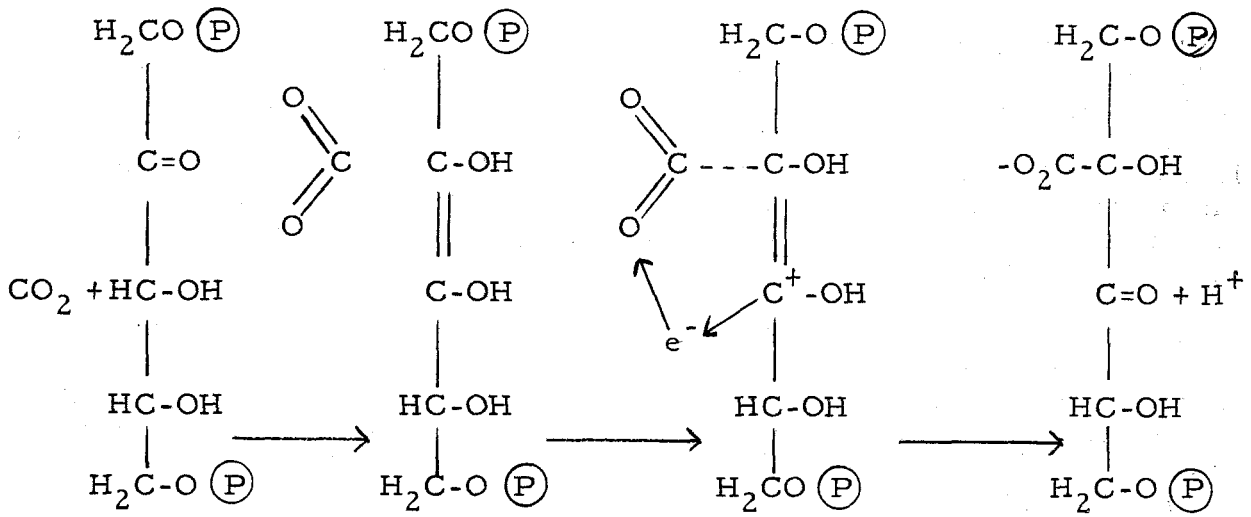
$$2\,H_2 + O_2 \longrightarrow H_2O$$

to help accomplish the reduction reaction

$$2\,H_2 + CO_2 \longrightarrow (CH_2O)_x + H_2O.$$

Since the free-energy change for the latter reaction as written is negative, it appears that the presence of a stronger oxidizing agent than CO_2 is required to produce some component or reagent necessary to permit the reaction to proceed. This reagent may be ATP, and the oxidant required may be either O_2 or another oxidant produced photochemically. It is the reaction of this oxidant (or O_2) with the activated (H_2) which can produce ATP by the mechanism almost certainly similar to that ordinarily known as "oxidative phosphorylation." Under special conditions such as these, then, in which a high level of both H (from H_2) and ATP are simultaneously present, may we expect the photosynthetic carbon cycle to operate as it seems to.[53]

The precise detailed mechanism of the carboxylation reaction is not yet known with certainty. The first step may be the enolization of the ribulose diphosphate to form a double bond between carbon atoms Nos. 2 and 3. An addition of the carbon dioxide may then take place with the shift of an electron from the hydroxyl hydrogen on Carbon 3 to one of the carboxyl oxygens. This hydrogen would come off as hydrogen ion, leaving a carbonyl group at Carbon 3. The resulting compound would be a β-keto acid.



Once formed, the β-keto acid appears to undergo "acid splitting" into two molecules of PGA. This is analogous to the splitting of acetoacetate to two molecules of acetate. "Ketone splitting" would produce CO_2 and a 3-keto pentose. Other possibilities are hydrogenation of the carbonyl group followed by a rearrangement, which would result in one molecule of PGA and one molecule of phosphoglyceraldehyde; or a direct splitting or "reverse benzoin" type reaction, which would result in the formation of one molecule of phosphoglyceraldehyde and one of 3-phosphohydroxypyruvic acid.

Since PGA is known to be one if not the only product of the carboxylation reaction in vivo, it is necessary to consider only the "acid splitting" and reductive splitting. There was considerable evidence for the acid split leading to PGA only, even before the enzyme was studied in vitro. Studies were made of the change in concentrations of RuDP and PGA that occur in algae immediately after turning off the light.[18] (also Bradley, private communication.) It was found that the concentration of PGA rose very rapidly under these conditions while that of RuDP dropped very rapidly. This was explained as resulting from a cessation in the reduction of PGA, due to the sudden decrease in photochemically formed reducing agent, but at the same time, a continuation of the carboxylation of RuDP leading to the formation of PGA. The latter reaction, therefore, was not apparently affected at once by the lack of illumination. This result indicates that the formation of PGA from RuDP and CO_2 does not necessarily involve a reduction, although it is possible to postulate for this reaction a reducing agent with a longer half life in the dark than the reducing agent required for the reduction of PGA.

Finally, recent work in this laboratory (J. Mayaudon, private communication) with somewhat more purified enzyme preparation, C^{14}-labeled RuDP, and CO_2, indicates that the only product of the carboxylation reaction in vitro is PGA.

Despite these arguments, there still remains some possibility that the first product of the carboxylation reaction, the six-carbon β-keto acid, might undergo different fates in the light and in the dark. In the dark, splitting to two molecules of PGA would proceed as discussed above; but in the light, with

reducing agent (TPNH or DPNH) in plentiful supply, the β-keto acid might be reduced and then split by a rearrangement to one molecule of PGA and one of phosphoglyceraldehyde. This would provide a route for the formation of one molecule of triose phosphate without the requirement of an ATP molecule. This possibility should be kept in mind during the following discussion of the energy requirements of the carbon-reduction cycle, which is based on the assumption that each molecule of triose phosphate formed requires the supply of a molecule of ATP.

Estimation of the free-energy change for the reaction of RuDP with CO_2 and water to produce two molecules of PGA showed a net free-energy change at physiological conditions which was either zero or negative,[4] indicating that the reaction would proceed under the influence of a suitable catalyst without energetic coupling with some other reaction. It thus appears that energy is supplied to the cycle for the reduction of carbon in the form of reduced CO II (TPNH) and ATP, both in the reduction of PGA to phosphoglyceraldehyde and ATP in the phosphorylation of RuMP. For each carboxylation, using one molecule of CO_2 and producing two molecules of PGA, two TPNH molecules and two ATP molecules would be converted to DPN^+ and ADP + PO_4^- in the subsequent reduction of PGA to phosphoglyceraldehyde. The enzymatic formation of RuDP from ribulose monophosphate has been shown by Weissbach et al.[63] to require a molecule of ATP. Since the enzymatic conversion of triose phosphate to ribulose monophosphate probably does not require the expenditure of any ATP or other substances that supply energy through energetic coupling reactions, the net supply of energy to the carbon reduction cycle for each molecule of CO_2 reduced is that required for the formation of two molecules of reduced TPN and three molecules of ATP as follows:

$$2(TPN^+ + H_2O \longrightarrow TPNH + H^+ + 1/2\ O_2), \qquad \Delta F = +103 \text{ kcal};$$

$$3(ADP + PO_4^{=} \longrightarrow ATP), \qquad \Delta F = +32 \text{ kcal};$$

which adds up to 135 kcal. (If one molecule of triose phosphate is obtained without consuming ATP, then the total requirement is 2 TPNH and 2 ATP molecules, or 124 kcal.) Since the energy required for the reaction

$$CO_2 + H_2O \longrightarrow (C_6H_{12}O_6)_{1/6} + O_2$$

is about +116 kcal, about 19 kcal apparently have to be expended to make the cycle operate at a high rate. The 135-kcal (or 124 kcal) requirement, on the other hand, represents the absolute minimum of energy that must be obtained from light, exclusive of any losses in the photochemical and other energy transfer reactions. We shall return to a discussion of the energy requirements for these other processes later.

The relation between photosynthesis and respiration is shown in Figure 2, where respiration is indicated by dotted lines and photosynthesis by solid lines. The points at which reducing agents and ATP are utilized in photosynthesis or produced in respiration are indicated.

It can be seen that the path of carbon reduction in photosynthesis is far

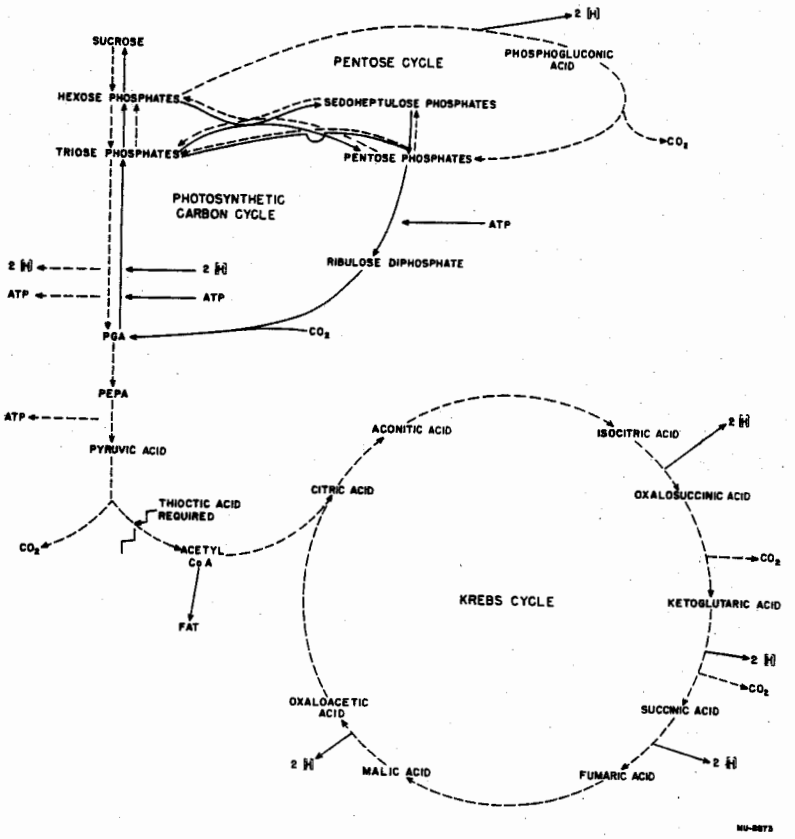


Fig. 2. The relation of carbon paths in photosynthesis and respiration.

from a simple reversal of its path in respiration. However, the conversion of PGA to hexose in photosynthesis and the reverse in respiration appear to follow similar if not identical paths. Moreover, some of the pentose-cycle path seems to have some steps in common with photosynthesis.

THE LIGHT REACTION

In considering the most characteristic reaction of photosynthesis, the light reaction, it is necessary to keep in mind the physical arrangement of the chloroplast structure as it is now thought to exist. The fine structure of the chloroplast (or of the grana into which some chloroplasts seem to be divided) is believed to be laminar, with very thin-- perhaps monomolecular -- layers of chlorophyll alternating with thicker layers of proteins and lipoproteins. While the organization and thickness of these layers seem to vary with the species, --and more in some algae than in higher plants, it seems likely that the electrochemical fields that exist at the chlorophyll-protein and lipoprotein interfaces are similar in all cases. Thomas[56] has recently studied the Hill reaction in particles of sublaminar size. Measurement of oxygen evolution as a function of the particle size showed an ability of particles as small as 10^6 Å^3 in volume to carry out the Hill reaction with about 50% the specific activity of intact grana, but a rapid decrease in such ability with smaller particles, and no activity with particles with volumes of less than 2×10^5 Å^3 at a given light intensity. Higher light intensities, however, resulted in Hill-reaction activity with even smaller particles, so it was concluded that if there is a physical unit of about 10^6 Å^3 volume, or 100 Å diameter, it is capable of producing oxygen in the Hill reaction even if partially fragmented.

Earlier, Milner et al.[42] using the photochemical reduction of 3, 6-dichlorobenzene indophenol as a measure of activity, studied the Hill reaction with particles of subgranar size, and obtained activity with a mixture of particles, the majority of which were about 20 Å in diameter. The same workers[43] after measuring Hill-reaction activity with these particles (about one-fourth that of intact chloroplasts), found that the particles could be aggregated, by precipitation with a variety of salts in the presence of 15% to 20% methanol, in such a way as to produce particles with increased activity. Loss of lipid material resulted in loss of activity.

These experiments indicate that there are physical units, about 100 Å in diameter and containing about 200 chlorophyll molecules, which are capable of carrying out the Hill reaction nearly as efficiently (~60%) as intact chloroplasts. If these units are broken down further, Hill reactivity falls off rapidly, but is present to some extent with considerably smaller particles, especially light intensities are high. Moreover, much of the original activity can be restored by reaggregation. The requirements for photo-decomposition of water with chlorophyll seem to be some aggregation of chlorophyll, lipids, and protein. Rodrigo[51] has studied associations of a few molecules of chlorophyll, finding no shift of the red-absorption peak to 6800 Å. However, when chlorophyll was mixed with some ground-up leaves that contained no chlorophyll initially, some shift of the red-absorption peak toward 6800 Å and some oxygen evolution in light with quinone were observed. The implication of this result seems to be that a degree of aggregation of the chlorophyll molecules sufficient to shift the red peak as

far as 6800 Å is required before the Hill reaction can function. It is interesting to note in this connection that the absorption spectra of chlorophyll in crystals of varying size formed from ethyl chlorophyllide in acetone has been studied by Jacobs and Holt[37] and a shift to the longer wave lengths with increasing crystal size was observed. A maximum shift of the red peak to about 7450 Å was found, beyond which there was no further shift with larger crystals. This shift is ascribed to resonance interaction between identical chromophores or migration of the resonance energy through the array.

The phenomenon of energy migration through the pigment aggregate brings us to a consideration of the light-absorption process. According to present views[24] it appears that most of the energy absorbed by plant pigments for subsequent conversion to chemical energy is either absorbed directly by chlorophyll a or transferred to chlorophyll a. The excited state is presumed to differ in energy from the ground state by only about 40 kcal/mole, corresponding to 6800 Å light, the longest-wave-length light that brings about photosynthesis with high efficiency. It is postulated that the extra energy that is absorbed at shorter wave lengths, either by chlorophyll a or other pigments, is converted to vibrational energy and eventually lost as heat. Thus the course of energy transfer beyond chlorophyll would be unaffected by the wave length of the light absorbed. It has long been known, in fact, that the yield of oxygen evolved per quantum of light absorbed is as high for red light as at any other wave length.

On the other hand, light absorbed at wave lengths around 4800 Å produces a relatively lower quantum yield indicating that pigments which absorb in that range may transfer their energy to chlorophyll inefficiently or may transfer some of their energy to other chemical reactions inefficiently. In the latter case, the course of subsequent steps in photosynthesis should be to some extent affected by the light energy converted to chemical energy without passing through the excited-chlorophyll-a stage. Such an effect has recently been reported by Voskrenskaya,[59] who has studied the products of carbon reduction, using C^{14}, as a function of the wave length of the incident light. He reports an enhanced ratio of protein to carbohydrate in blue light as compared with red light. This effect seems to be more pronounced at longer periods of time than those required for the early steps in the reduction of carbon, described earlier. It seems likely that such effects are due to changes in the relative rates of transformation for various photosynthetic intermediates into other substances. These changes in rates of specific reactions are probably photocatalytic, and the light energy is used only in the activation or deactivation of an enzyme.

Another photoactivation has been reported recently by Warburg.[61] In this case it was found that the manometrically measured quantum yield with either red or green light was greatly affected by catalytic amounts of added blue light. Some rather special conditions for the culturing of the algae used in the measurements appear to be necessary for this effect to be seen, since in other experiments reported by Warburg, high quantum efficiencies were obtained with red light only, so that the role of the blue light again appears to be in the activation of an enzyme -- but in this case, one which effects the efficiency of the energy-conversion path. Another possible interpretation of this effect is presented in the section on the quantum requirement.

The step in photosynthesis which is perhaps most characteristic is the efficient conversion of energy of an excited state of chlorophyll a to the stored energy of new chemical bonds. The first point to consider is the quantity of energy actually available for transformation. It has been frequently proposed that the primary excited state of chlorophyll a has such a short half life (10^{-13} sec) that direct conversion of the electronic energy of this state to some chemical reaction, or the transfer to some other pigment, might not take place before the energy was lost by fluorescence. Consequently, it was believed that transition to a metastable triplet state with concurrent loss of some of the electronic energy must first occur, followed by conversion or transfer of the energy of the triplet state. According to Scheibe,[52] however, transfer of electronic energy in a condensed pigment system can occur in 10^{-14} sec. It seems possible, therefore, that in the aggregated chlorophyll-lipid-protein system, the full 40 kcal may be transferred to a suitable proximate acceptor, at least as far as competition with fluorescence is concerned.

The conversion of electronic energy to chemical energy may involve either (a) the transfer of electronic energy to some acceptor molecule other than chlorophyll, followed by energy conversion, or (b) the transfer of an electron from the chlorophyll aggregate (array) to some other molecule, and a corresponding recovery of an electron by the positively charged chlorophyll-molecule group from some other source. Either of these types of conversion seems possible at the present time, and we consider both.

In order to accept electronic energy from chlorophyll and convert it to chemical energy, a compound, which could be called the quantum converter, should possess several properties. It would have to be closely associated with, or incorporated in, the chlorophyll aggregate, and should possess some state differing from its ground state by about 30 to 40 kcal in order to accept the energy of a quantum from the chlorophyll. It would convert this energy by some process that occurs in a time that is short compared to the time required for the return of the energy to chlorophyll or its dissipation in a nonuseful way, and in so doing would form new chemical configurations, which would store most of the energy received from the chlorophyll. By "new chemical configurations" we mean only that some nuclei would have moved sufficiently to prevent the loss of the received energy either by its return to the donor or conversion to heat; that is, the energy would now be trapped in a new configuration that could not return easily to the former one.

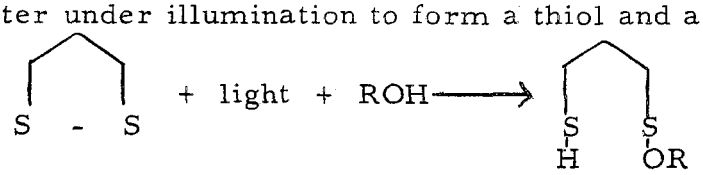
When the energy had undergone this conversion, the quantum converter, in its new form, would pass the energy on. Since, in this picture, the most energetically difficult step -- the photolysis of water -- has yet to occur, we suppose that the new form of the quantum converter would then react with water to produce a reducing agent and some form of hydroxyl or peroxide compound which can ultimately liberate oxygen. A requirement for this reaction is that the resulting bond energies plus about 30 to 40 kcal be about the same as the bond energies of broken bonds, if the reaction is to provide a means of efficient energy conversion. The products of the reaction should not be able directly to recombine easily in such a way as to produce water again (back reaction), but should be able to react

separately to produce ultimately oxygen and reducing power. Finally, the quantum-converter molecule should be able to return to its original state, after having transferred its electrons (reducing power) and oxygen to other molecules.

In addition to the requirements imposed by the mechanism, there is the necessity that the quantum converter be present in sufficiently high concentration in the chloroplast to account for the observed rates of quantum conversion, both in steady-state photosynthesis and in flashing-light experiments. This requirement of concentration depends, of course, on the time required for the quantum converter to undergo one cycle, from acceptance of the quantum of electronic energy to its return to its original state.

Thioctic acid (pyruvic acid oxidase, lipoic acid) has been proposed as a compound that may satisfy all the above requirements.[16, 3] It was suggested that, following the absorption of a quantum by chlorophyll, this energy is transferred to thioctic acid, causing the S-S bond of the latter molecule to break to give a diradical. It was postulated that this diradical then reacts with water, forming a sulfhydryl and a sulfhydroxyl group. Dismutation of this reaction product results in a dithiol molecule and a disulfenic acid. The dithiol would then reduce DPN or TPN and would itself be reoxidized to thioctic acid, while the disulfenic acid would undergo a series of reactions resulting in the reformation of thioctic acid and the liberation of oxygen. All these reactions from the dismutation onwards would probably involve catalysis by metalloproteins.

The lipophyllic properties of thioctic acid and its small molecular size would permit close association with the chlorophyll aggregate and might account for the apparent lipid requirement of the Hill reaction. The formation of the diradical by breaking the strained ring has been suggested as the mechanism for storing the accepted electronic energy. Estimates of the bond energy for the S-S bond in simple open-chain molecules ranging from 50 to 70 kcal, together with estimates for the reduction of the dissociation energy of the S-S bond due to ring strain in the 6, 8-trimethylene disulfide ring of 10 to 25 kcal, indicate the possibility that formation of the free radical could store 30 to 40 kcal.[3] A number of studies with model systems (6, 8-trimethylene disulfide in uv light) were presented which indicated, among other things, an ability of the trimethylene disulfide to react with ROH (alcohol) or water under illumination to form a thiol and a sulfenic acid or ester.



Two pieces of biological evidence were offered to support the suggestion that thioctic acid participates in the quantum conversion. It had already been observed that in photosynthesizing plants the conversion of photosynthesis intermediates to Krebs-cycle intermediates is inhibited during illumination, but occurs rapidly in the dark. Since this conversion involves the formation of acetyl CoA from pyruvic acid, a reaction which requires thioctic acid as

a cofactor,[14, 33, 44, 48, 49] it had been suggested[18] that this reaction is blocked in the light because most of the thioctic acid is maintained in the reduced dithiol form within the chloroplast. The reduction of thioctic acid could occur, of course, as a secondary reaction resulting from the formation of some other primary reducing agent in the light-energy conversion. However, the favorable characteristics for quantum conversion which the thioctic acid possesses, together with the indication that it was reduced in the light, led to the supposition that it might be in the primary reaction.

When algae are allowed to take up added thioctic acid in the dark for several minutes and then killed with quinone and the Hill reaction is studied in the light[9] it is found that the initial rate of oxygen evolution is increased by as much as 50% over the rate observed in the control in which no thioctic acid is added. This result could be interpreted as evidence for the participation of thioctic acid in the primary conversion step or as an acceleration of a dark-reaction transfer of reducing power to quinone. Thus the biological evidence for the role of thioctic acid as a quantum converter is suggestive but not unequivocal. However, since the oxygen evolution in the Hill reaction is insensitive to inhibition by parachloromercuribenzoate, a more limited role of thioctic acid in which it acts only as an acceptor of an electron from chlorophyll seems more likely than the above role, in which it removes this electron from oxygen after receiving energy from excited chlorophyll.

Of the various possible chemical reactions of chlorophyll under the influence of light we consider only the transfer of an electron. Since there is evidence that the light-absorption process functions with chlorophyll in an aggregated system, it is interesting to consider -- instead of the reaction of a single chlorophyll molecule -- the possibilities that exist with some sort of orderly array of chlorophyll molecules. This array is probably not actually crystalline chlorophyll, but may well be an orderly arrangement of chlorophyll molecules associated with other molecules and protein or lipoprotein. We may think of the electronic system of such an aggregate as a single unit in which the π-electrons of the chlorophyll molecules interact. The absorption of an electromagnetic quantum raises one electron of this system from the ground state, in which it is confined to a single molecule, into a state in which it may migrate throughout the array, i.e., into a conduction level. If there is built into this structure a permanent polar character such as exists at a "p"-"n" junction, for example, these photoconduction electrons diffuse toward the positive end of the permanent dipole, leaving a positive hole to diffuse in the opposite direction.

Thus a separation of charge is induced by the light, which may be neutralized by a suitable electron acceptor at one end and a corresponding electron donor at the other end to drop electrons into the positive holes that have been photocreated. In order to complete the separation of the electron and the positive charge and make use of the electrical energy available, all that is needed is either a semiconductor that transmits only electrons or positive charges, or else an acceptor molecule that can accept either an electron or a positive charge (i.e., contribute an electron) or both, in such a way as to produce an irreversible change.

The possibility of a semiconductor is an interesting one in that it provides

a possible function for the lipid constituents. One might consider most of the lipid material as an insulator with occasional conductor molecules to pass electrons through. Such a function might be served by a conjugated molecule like a carotenoid, which could accept an electron at one end and give an electron to a suitable acceptor at the other end. At the same time, the positive charges left behind would be reacting with water, probably through the agency of some metalloprotein, to produce oxygen. The electron acceptor, in the Hill reaction, could be the supplied oxidant such as quinone, while in photosynthesis the acceptor could be the primary carrier of reducing power (thioctic acid).

The conductor function for a carotenoid compound might explain the stimulation of photosynthesis by catalytic amounts of blue light observed by Warburg.[61] Warburg has suggested the participation of carotenoid somewhere in the transfer of electrons between the photochemical reaction and the reduction of carbon dioxide as an explanation of the blue-light effect. It should be noted, however, that Stanier (Roger Stanier, private communication) has studied mutants of Rhodospirillum which contain no carotenoids but which nevertheless are able to carry out reduction of carbon dioxide during photosynthesis.

The advantage of the semiconductor arrangement would be the physical separation of the points at which reducing agent and oxidizing agents are formed. Also the nonspecificity of oxidants required for the Hill reaction could be explained, since they could be reduced directly by electrons supplied by the photoactivation of chlorophyll rather than by some specific primary reducing agent. During actual photosynthesis, a specific reducing agent would undoubtedly be formed and would provide a more efficient transfer of the energy available from the electrons obtained from the photochemical reaction. We would expect that this compound would have special properties that would particularly suit it to the task of accepting electrons and, in its reduced form, transferring energy to the carbon-reduction apparatus. It might be expected that this compound would stimulate the Hill reaction, owing to its special qualifications for receiving electrons. For example, if thioctic acid were the compound receiving the electrons from the chlorophyll during photosynthesis, it might be expected that thioctic acid would stimulate the Hill reaction under suitable conditions even though it is not required for the Hill reaction to function.

Such a stimulation has been observed by Bradley,[9] who studied the rate of oxygen evolution in the Hill reaction as a function of added thioctic acid. When quinone was added as an oxidant at a concentration that produced the highest rate of evolution of oxygen with quinone alone, it was found that the addition of thioctic acid in a molar concentration only 0.1 as great as that of the quinone produced a 35% stimulation of the initial rate. Moreover, this stimulation was observed with 6,8-dithiooctanoic acid (6-thioctic acid), whereas 5-thioctic acid and 4-thioctic acid were not effective. The reduced form (dithiol) of thioctic acid and the more oxidized form (the sulfoxide) were also found to be ineffective. It appears that, barring some enzymatic specificity which seems unlikely in the Hill reaction, the special property of 6-thioctic acid that is responsible for its activity is the ring strain in the five-membered ring which permits the more ready breaking of the

sulfur-sulfur bond.

One advantage of considering the chlorophyll as an aggregated system is that it permits a more reasonable mechanism for the transfer of one electron at a time, with each electron requiring one quantum of light energy. This corresponds to the well-known four-quantum theory in the primary step, since the transfer of four electrons, requiring four quanta, is required to form one molecule of O_2.

Reactions between a single molecule of chlorophyll, water, and hydrogen or electron acceptor are difficult to formulate, since one is faced in that case with the necessity either of absorbing two consecutive quanta in one chlorophyll molecule in order to have enough energy to form oxygen and reducing agents of the strength of TPNH (about 51 kcal), or else of forming oxygen and a reducing agent of insufficient strength which could be used in subsequent reactions involving dismutations of energy to form better reducing agents. Another alternative is that a single molecule of chlorophyll, activated by one quantum, reacts with some activated, hydrated compound in which the O-H bond could be more readily broken, and thus has left over enough energy to form a good reducing agent. It may well be that water is in fact incorporated into some compound to weaken the O-H bond before the O-H bond is broken, regardless of the mechanism of electron transfer, but it is doubtful if this activation is sufficient to permit the formation of oxygen and reducing power of the strength of TPNH at the same time by one quantum.

Recent proposals involving reactions in which one molecule of chlorophyll provides both electrons in a given H_2O photolysis include the one by Levitt,[39] who suggests that a chlorophyll molecule, excited by one quantum of light, gives up an electron to thioctic acid forming oxidized chlorophyll, after which the molecule of oxidized chlorophyll absorbs a second quantum and transfers a second electron. The resulting dipositive chlorophyll then reacts with water to produce hydrogen ion and oxygen. In the meantime, the thioctic acid has been reduced by the two electrons to the dithiol.

On the other hand, Wessels[64] has proposed a one-quantum-per-two-electrons reaction in which a much weaker reducing agent, reduced Vitamin K, is produced. This reducing agent is then used in part to produce ATP, and in part to produce a reducing agent of the level of TPNH through the expenditure of ATP in a coupled reaction. The formation of reduced Vitamin K and oxygen from water and Vitamin K is said to require 39 kcal, so that the energy of one quantum would have to be used with nearly 100% efficiency. Such an efficient mechanism will be especially attractive if the very low quantum requirements reported by Warburg prove correct. The presence of Vitamin K in chloroplasts and its concurrent formation with chlorophyll[23] are also favorable to this suggestion. Finally, its oxidation-reduction potential is close to that measured with illuminated chloroplast preparations.[50] The mechanism proposed by Wessels for the formation of ATP from reduced Vitamin K is reasonable but energetically very inefficient in that only one ATP is formed for each molecule of reducing agent used. No specific mechanism was proposed for the formation of TPNH from TPN^+ by the oxidation of reduced Vitamin K and the conversion of ATP to ADP and inorganic phosphate, but this reaction would be a bit uphill energetically,

since the difference in redox-free energies between TPNH and reduced Vitamin K is apparently about 12.5 kcal, slightly more than the 10 or 11 kcal now thought to be available from ATP hydrolysis. Besides this, the proposal of Wessels requires that the steps in the liberation of O_2 from whatever intermediates may be formed in the reaction of oxidized chlorophyll with water proceed with virtually no change in free energy. In other words, this proposed mechanism includes one very inefficient step and a number of steps that are nearly 100% efficient in energy transfer. While this is entirely possible, we find it slightly more satisfying, from a thermodynamic viewpoint, to suppose that most of the steps involved in the energy transfer from one system to another proceed efficiently but with a small loss of energy in each, thus providing a smooth driving force throughout the entire process which will not require the enzymes at any stage to cope with infinitesmal concentrations of substrates.

INTERMEDIATE TRANSFER SYSTEMS

We have already anticipated, in the discussion of both the carbon-reduction cycle and the light reaction, some of the reactions involved in the transfer of reducing power and energy from the light reaction to carbon reduction, and in the evolution of oxygen from whatever products are formed in the breaking of the O-H bond. Assuming, for the time being, that the theories above, which require the absorption of a quantum for each electron taken from water (whether by quantum conversion by thioctic acid or by transfer of an electron by the chlorophyll aggregate from water to reducing agent), are correct, there is ample energy in four quanta of 6800 Å light (168 kcal/mole) to bring about the photolysis of two water molecules and the formation of two molecules of reducing agent of strength equal to TPNH:

$$2\left[H_2O + TPN^+ = 1/2\ O_2 + TPNH + H^+\right], \qquad \Delta F = +103 \text{ kcal.}$$

The entire excess of energy, some 65 kcal minus whatever was lost in the primary absorption and conversion processes, is available for the evolution of oxygen from the intermediates formed from the oxidation of water. It is possible that some of this energy may be used in the formation of some ATP from ADP and inorganic phosphate. Whether or not this occurs is very important in the evaluation of possible quantum requirements. We have already arrived at a quantum requirement of two molecules of TPNH and three molecules of ATP for each molecule of carbon dioxide reduced. If one molecule of the primary reducing agent must be oxidized in order to form two or three molecules of the required ATP by some reaction similar to that which couples the energy of the oxidation of DPNH to the formation of ATP,[38] then the total requirement for equivalents of reducing agent will be seven or six, requiring seven or six quanta.[4] If all the ATP molecules could be supplied from the energy left over from the evolution of oxygen as suggested above, then the quantum requirement would obviously be only four. The decomposition of water requires by far the greater portion of the energy available from the primary photochemical reaction. From the redox potential of the primary reducing agent, which in our scheme must be about 0.3 v, we can say that the energy stored by the transfer of the electron to the reducing agent is about 7 kcal/mole (taking that in H_2 at one atmosphere in contact with 1 N H^+ as zero). This leaves

a remainder, from a 42-kcal/mole-quantum, of about 35 kcal. If there is a loss of about 5 kcal in the transfer of the electron from the chlorophyll aggregate, there is left about 30 kcal/mole to be stored in each positive charge ("hole"). This would then be a potential of about 1.3 v. The potential required for the half reaction

$$2\,H_2O = H_2O_2 + 2\,H^+ + 2\,e^-$$

is about 1.2 v at pH 7 and 10^{-6} M H_2O_2. Thus the reaction will go as written provided that the very high activation energy required for the removal of electrons from water-oxygen atoms, which would result in formation of hydroxyl radicals in solution, can be overcome. We may suppose that the hydration of a suitable surface on the granar fragment, perhaps resulting in actual hydrated compounds, results in an orientation of -OH groups which permits the formation of O-O bonds concurrently with the removal of the electrons from the water.

The formation of the positive and negative potentials discussed above requires some mechanism for obtaining just the right distribution of energy to achieve the necessary oxidation and reduction. This can be accomplished by extending somewhat further the proposal for the separation of charges through the agency of semiconductors. We may think of the subgranar unit as a photoelectric battery. The driving force for this battery is the light energy absorbed by the chlorophyll. The absorption of light produces in the aggregate conduction electrons and their corresponding positive "holes." This part of the structure can be considered a conductor after light absorption. On either side of the chlorophyll aggregate is a layer of semiconducting material. This material may be lipid or lipoprotein. One layer contains a permanent structural excess of electrons ("n" volume), permitting the conduction of positive charges. The other layer contains a permanent structural deficiency of electrons ("p" volume), permitting the conduction of electrons. With the creation of mobile charges by light absorption there follows a flow away from the chlorophyll aggregate and across the semiconducting layers. If the potential between these two layers could be measured, it would be found equivalent to ~40 kcal/mole minus the 5 kcal/mole loss postulated above, or 1.6 v. If the circuit between these two "electrodes" is completed by chemical reactions, as it is in photosynthesis, the potential at each electrode, relative to the ground state of the system, is simply that required by the oxidants or reductants with which the electrons and positive charges must react. Thus, in terms of our arbitrary "ground," which is the standard hydrogen electrode potential, the 1.6 v potential across the "battery" is distributed as 1.3 v positive and 0.3 v negative, since these are the potentials, relative to our "ground," with which the electrodes must react. Viewed in this way, it appears that the recently developed solar battery[22] may have been preceded by a similar but much more efficient process in photosynthetic organisms!

The evolution of oxygen from hydrogen peroxide according to the reaction

$$H_2O_2 = 1/2\,O_2 + H_2O$$

proceeds with a positive potential of 0.37 v for 10^{-6} M peroxide, so that

another 8.5 kcal/mole of excess energy is expended per electron. There is some question whether this reaction could be catalyzed by catalase,[55] owing to the apparent lack of inhibition of the Hill reaction by cyanide. This reaction can be catalyzed, however, by a number of simple inorganic compounds, so perhaps this problem is not too serious. Moreover, inhibition of oxygen evolution in the Hill reaction by orthophenanthroline has been observed,[29] so some iron-containing enzyme may be involved. Finally, the recent work of Chance[21] has demonstrated the formation of catalase-H_2O_2 complexes under aerobic conditions. It appears that this complex can form with concentrations of H_2O_2 as low as 10^{-8} M.

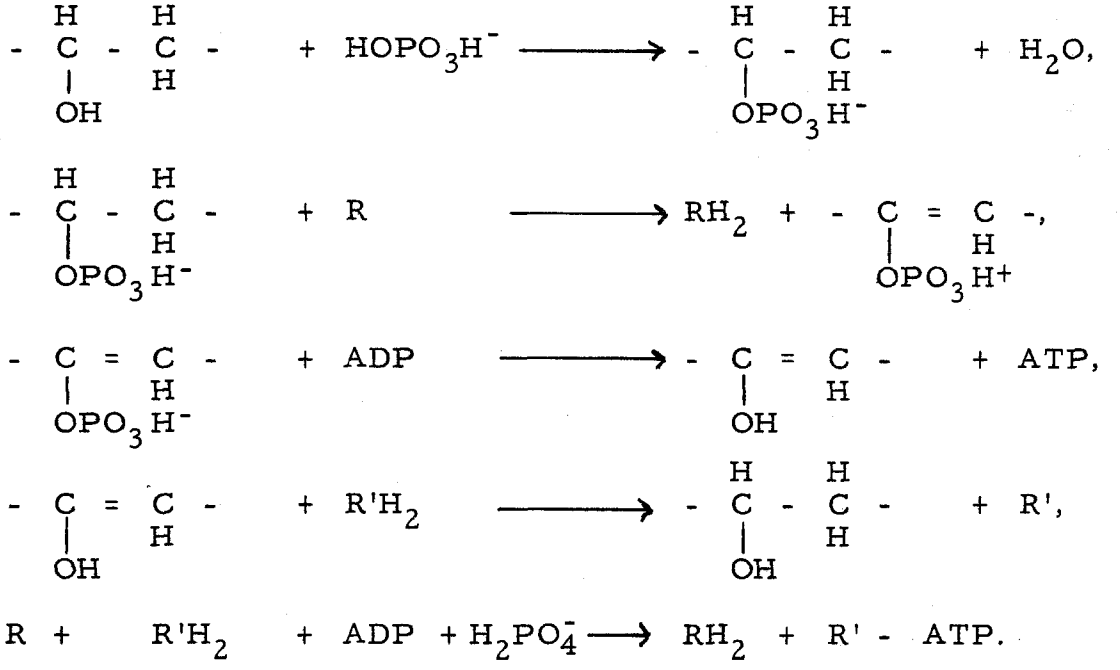
Consideration of the above mechanism of oxygen evolution suggests that the place where energy may possibly be available for use in forming ATP, or perhaps additional reducing agent, is in the liberation of oxygen from peroxide. Some 17 kcal are available from each molecule of peroxide. However, it is difficult, with only our present knowledge, to visualize the possible mechanism of this energetic coupling. On the other hand, it is attractive to suppose that the peroxide may be used in part to oxidize some ferrocytochrome[19] in a reaction catalyzed perhaps by peroxidase.[19] Thus the requirement for an oxidizing agent to react ultimately with the primary reducing agent could be met without requiring oxygen. This is rather useful because of the evidence that molecular oxygen is not required for photosynthesis. Allen[1] has reduced the concentration of molecular oxygen in contact with photosynthesizing organisms to a value of 0.004 mm Hg and finds, at this level, no diminution of the rate of photosynthesis. The absence of a back reaction involving molecular oxygen was also shown by the studies of Brown[10] with the mass spectrometer and isotopically labeled oxygen.

It is interesting to suppose that the ferrocytochrome oxidized by H_2O_2 is reduced cytochrome f, discovered by Hill and Scarisbrick.[35] It was found that this compound was present in considerable amounts in green chloroplast material and that it had an oxidation-reduction potential of +0.365 v, about 0.1 v better as an oxidizing agent than cytochrome c. Since the oxidizing potential of H_2O_2 for the physiological conditions as given above is +1.2 v, there is a potential difference between these two half reactions of 0.8 v, or a negative free-energy change of 37 kcal/mole for the oxidation of two reduced cytochrome f molecules with one H_2O_2 molecule.

The oxidized cytochrome could then react with other electron carriers, perhaps other cytochromes, and eventually with the primary reducing agent or with TPNH. Since the oxidation-reduction potential of the latter is now considered to be -0.324 v,[15] there is a potential difference between cytochrome and TPN^+ of 0.65 v, or a negative free energy of 30.3 kcal for the oxidation of one mole of TPNH by oxidized cytochrome f.

Very little is known about the mechanism of formation of ATP during the oxidation of TPNH. There is enough energy available in each of the steps suggested above to bring about the formation of two moles of ATP with ease. We can suppose that the general type of mechanism for the oxidative formation of ATP may involve esterification of a hydroxyl group with inorganic phosphate, dehydrogenation of an adjacent C-C or C-N bond with a suitable oxidizing agent to form a "high-energy phosphate group," a

reaction with ADP to form ATP and an unsaturated alcohol, and finally, reduction of the unsaturated alcohol to the original substance.



The $-CH_2-$ group of the reacting alcohol can also be -NH-. Considering the number of steps involved in the above mechanism, we could expect that at least 5 calories negative free-energy change would be required for the entire process, so that about 15 kcal/mole might be required for the formation of ATP by such a mechanism. It is thus possible that anywhere from 1 to 4 molecules of ATP might be formed for each molecule of primary reducing agent used, provided electron carriers of suitable intermediate potentials are available. What these may be is difficult to say, but some cytochromes, for example cytochrome b, lie intermediate between cytochrome f and TPNH. It is conceivable that there is still another type of process for the generation of ATP which differs fundamentally from the one outlined above involving the intermediate formation by oxidation of a "high-energy phosphate group" in the form of an enol ester or anhydride. This is suggested by the increasing knowledge of "oxidative phosphorylation" and the participation of metalloproteins in this process -- particularly metalloflavins and/or porphyrin -- together with the fact that tripositive (higher valence state) ions form stronger and more compact complexes than do dipositive ions (lower valence state). An example of the manner in which such a process might operate is shown in Fig. 3. The metal (in this case Fe) in its reduced form (Fe^{+2}) could bind into a complex the terminal phosphate of ADP and one orthophosphate ion. This complex, upon oxidation to the higher valence state (Fe^{+3}), would contract and induce the displacement of an OH^- from orthophosphate by the $-O^-$ atom of the terminal phosphate of ADP, thus producing the stable ATP chelate of the Fe^{+3}. In order that the ATP be liberated for other uses, the Fe^{+3} must be reduced again to Fe^{+2}, for which the chelation constant is much smaller. The cycle is thus complete, the net result being the transfer of an electron from the reducing agent to some oxidizing agent of higher potential via the Fe atom with the trapping of some (if not all) of the energy

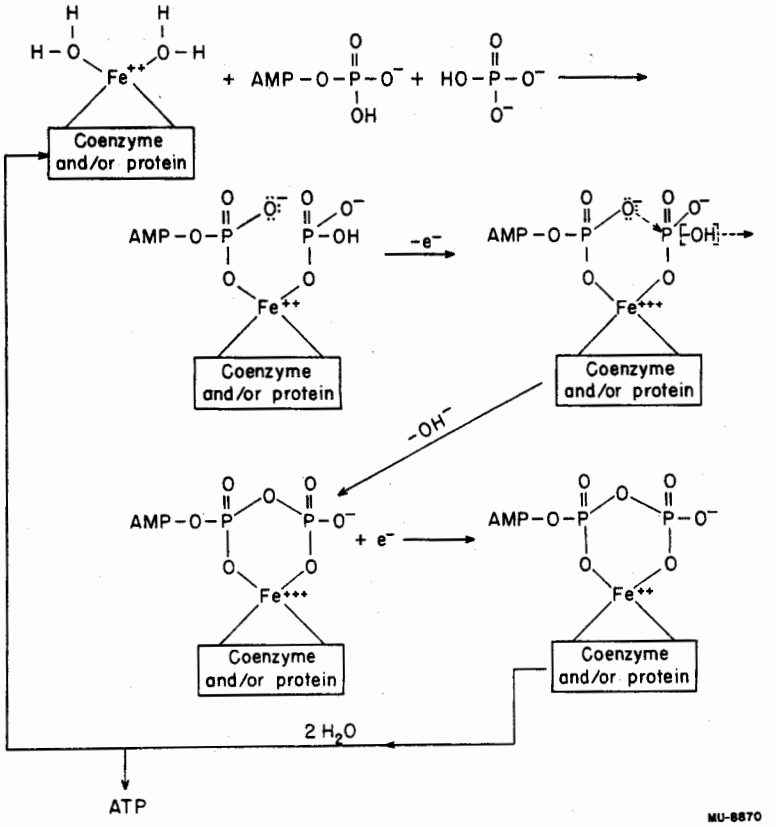


Fig. 3. A suggested mechanism for ATP formation via oxidation and reduction of a metalloenzyme.

of this transfer in the form of ATP. It is interesting to note that Chance[20] has observed an apparent requirement of the reduction step for the liberation of ATP in the course of oxidative phosphorylation.

The process postulated for the formation of primary reductant and H_2O_2 has a quantum requirement of four for each H_2O_2 formed or two for each RH_2 formed. The overall quantum requirement depends on the number of RH_2 molecules that must be used to form ATP. If all required ATP can be supplied from respiration reactions outside the chloroplast, as may be the case at very low light intensities, the over-all quantum requirement is four. At high light intensities the over-all quantum requirement will be 10, 7, 6, or 5, depending on whether the number of ATP molecules formed per RH_2 burned is 1, 2, 3, or 4 respectively.

The controversy regarding the minimal requirement of photosynthesis has not been settled. The recent experiments reported by Warburg[60] are very convincing, since quantum yields of four and even three are reported with high light intensities for long periods of time and without corrections for respiration, thus effectively answering criticisms based on the possibility that the reported quantum requirements are due to contribution of respiratory energy to photosynthesis.

This leaves only criticisms based on the evaluation of the manometric technique. No such evaluation is attempted here, but it may be worth while to point out one possible difficulty. Calculations of oxygen evolution and carbon dioxide uptake by the two-vessel method depend on the assumption of constant solubilities of these gases. However, the solubility of carbon dioxide may change significantly if the pH of the medium changes, and this in turn could be influenced by the secretion of acid by the algae. For example, it has been observed in this laboratory that in high light intensities algae produce glycolic acid. Tolbert (Nathan E. Tolbert, private communication) has found that glycolic acid formed in strong light by algae is secreted into the medium. One might speculate that perhaps blue light may activate some acid-secreting enzyme system though there is at present no evidence for this.

In view of this and many other difficulties inherent in the manometric determination of quantum yields, it has seemed desirable to try other methods of measuring oxygen liberation for quantum-requirement calculations. One such study is that of Brackett et al.,[8] who used a polarographic determination of oxygen and calculated a quantum requirement as low as six. A relatively simple and straightforward experiment has now been carried out using an oxygen analyzer employing paramagnetic measurement of oxygen.[5]

A suspension of algae was placed in a thin plastic cell of large area. A mixture of 4% CO_2 was passed through this suspension, then through an oxygen analyzer and a carbon dioxide analyzer. The circulation of gas through this closed system was accomplished by means of a pump. The indications from the analyzers were continuously recorded on a multipoint recorder. A uniform light field of 6300-Å light was obtained from a spiral neon tube with suitable filters, and incident and transmitted light intensities

were measured with a bolometer, that was frequently calibrated against three standard lamps. Small variations in the light field were mapped by a small photoelectric cell and suitable corrections made.

The measurements of photosynthetic rate were dependent only on the measured change in the percentage of oxygen in the system and the known volume of the system. Both of these are directly measured quantities which can be -- and were -- checked frequently with standard gas mixtures. Virtually no variation was found from time to time. The energy measurements were also simple and straightforward, since they involved essentially the measurement of energy absorption in a thin layer of large area.

The algae, Chlorella pyrenoidosa, were grown according to previously described conditions[6] in 4% CO_2. The quantum requirements of these algae were tested after a variety of preconditions. The best condition found was selected and determinations were made as a function of light intensity. The values of the quantum requirement were determined both for the uncorrected rate of photosynthesis and for the rate, which we will call photosynthesis, obtained by subtracting the dark-respiration rate from the uncorrected rate.

This correction seems justified in view of Brown's study[10] in which isotopic oxygen was used to demonstrate that no significant change in respiration of Chlorella pyrenoidosa rate took place during alternate fifteen- or twenty-minute periods of light and dark. The same paper, as well as the earlier ones by Emerson and Lewis,[25] Weigl et al.,[62] and Brackett et al.,[8] showed an increase in the dark-respiration rate (and the light rate as well in Brown's paper) that is produced by conditioning the plants with photosynthesis over that from leaving them in the dark for several hours. This indicates a photosynthesis enhancement of the rate of respiration over the rate required by the plants while resting in the dark.

If each of the experimentally determined quantum requirements is plotted against the ratio of photosynthesis to respiration (p/r), an interesting result is obtained. At high ratios of p/r, where it would be expected that respiration could contribute relatively little of the ATP required for photosynthesis, both corrected and uncorrected quantum requirements approach the same value, about 7.4. At low values of p/r, where respiration could contribute relatively more ATP, the corrected quantum requirement approaches four while the uncorrected quantum requirement becomes very great. This result, shown in Fig. 4, lends credence to the theory that the two molecules of primary reductant required for the reduction of one molecule of CO_2 are generated by 4 quanta but that when the ATP required for the reduction of CO_2 must be formed by reactions consuming the reducing agent, there is a net requirement of about 6 or 7 quanta for each CO_2 molecule reduced.

When the reduction in quantum requirement at low light intensities is multiplied by the total number of molecules of oxygen evolved, and when the product (number of quanta "saved" by respiration) is compared with the enhancement of respiration due to photosynthesis, it is found that about 7 quanta are "saved" for each extra molecule of oxygen taken up by photosynthesis enhancement of respiration over the resting dark respiration.

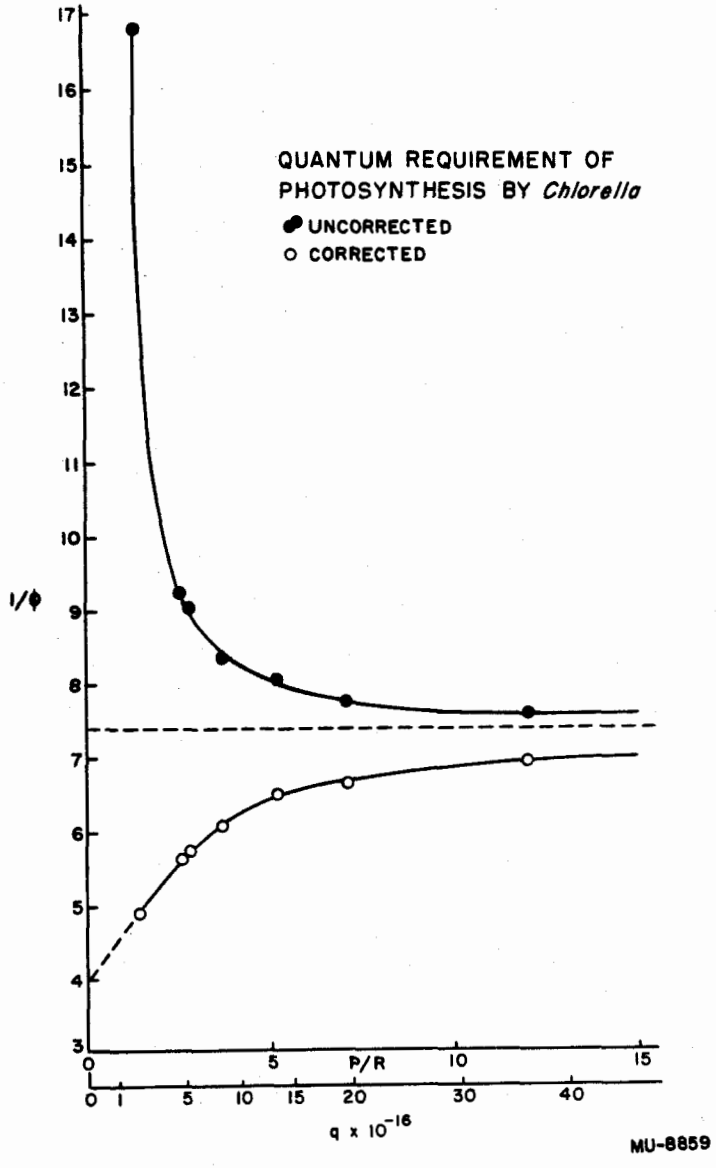


Fig. 4. Relation between quantum requirement in photosynthesis and the ratio of photosynthesis to respiration rate.

Since about 7 molecules of ATP are produced by each molecule of oxygen consumed in respiration, this result is consistent with the theory that respiration contributes energy to photosynthesis in the form of the reactivity of ATP and with the requirement, discussed earlier, of about 1 quantum for each molecule of ATP formed by burning photochemically produced reducing agent.

The relationships of energy transfer in respiration and in photosynthesis are shown in Fig. 5. It will be seen that in this scheme the principal "innovations" required for photosynthesis as compared with respiration are the photochemical "battery" and the use of perhaps two specialized electron carriers, thioctic acid and cytochrome f. As more is known about details of the energy-transferring processes, we may expect that additional special steps will be found.

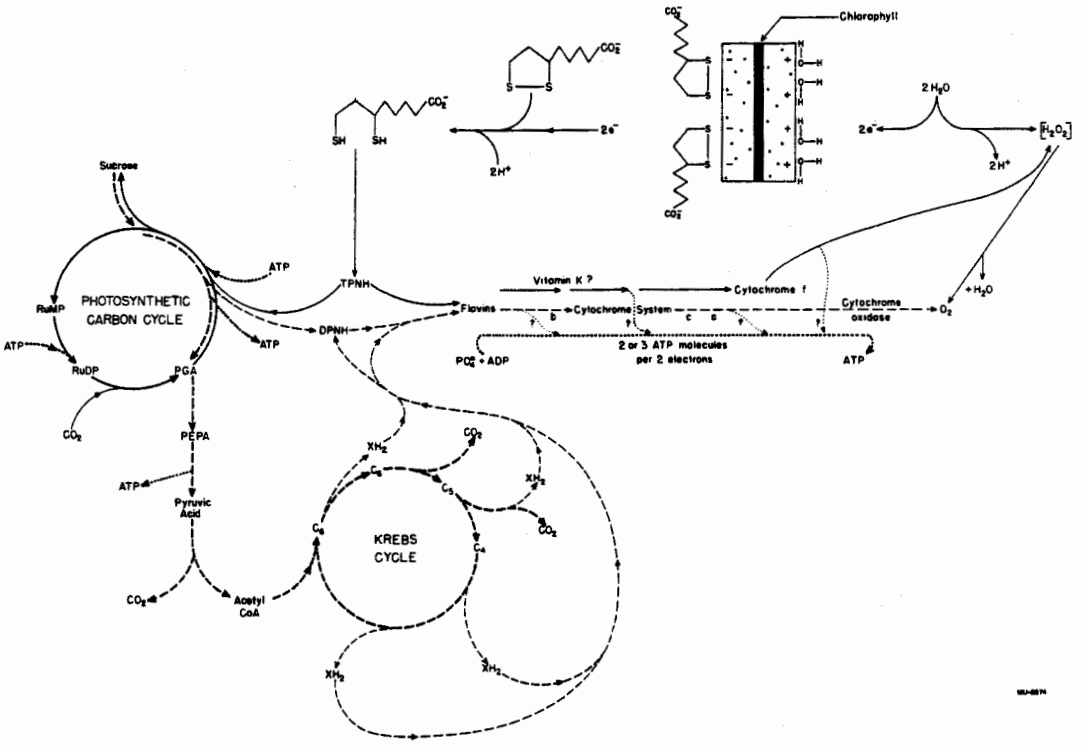


Fig. 5. Relations of carbon, phosphorus, and electron flow in photosynthesis and respiration.

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